

CLAIM LISTING

The listing of claims will replace all prior listings of claims in the application:

Claims 1-15 (cancelled).

16. (Currently Amended) A recombinant nucleic acid molecule encoding a modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195 of SEQ ID NO:3, or wherein said more than one amino acid substitutions occur at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 of SEQ ID NO:3, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity.
17. (Currently Amended) A recombinant nucleic acid molecule comprising the pneumolysin nucleic acid sequence of SEQ ID NO: 1 or variants of the nucleic acid sequence of SEQ ID NO: 1, said variants including changes in the nucleic acid sequence of SEQ ID NO: 1 that do not result in a change in the polypeptide encoded by the recombinant nucleic acid molecule ~~non-coding sequence changes thereof~~,
wherein said nucleic acid sequence comprises one or more of the nucleotide substitutions selected from the group consisting of:
A-50→G, G-54→T, T-181→C, A-196→T and T-302→C;
A-122→G, A-514→G, T-583→A and A-764→G;
A-187→T, T-380→A, A-382→C and T-443→A;

T-98→C, T-137→C, T-248→C, T-717→A and A-770→G;

T-134→C, A-305→G, A-566→G and T-583→G;

T-583→G;

T-583→A;

T-443→A;

and T-181→C.

18. (Previously Presented) The molecule of claim 16 or claim 17 as contained in a vector.
19. (Currently Amended) A ~~genetically engineered~~ microorganism or ~~genetically engineered~~ cell that has been transformed to comprise the recombinant nucleic acid comprising the molecule of any of claims 16-17 or claims 32-40.
20. (Currently Amended) The ~~genetically engineered~~ microorganism or ~~genetically engineered~~ cell according to claim 19, wherein the ~~genetically engineered~~ microorganism ~~or genetically engineered cell~~ is selected from the group consisting of bacteria[[,]] and yeast, and the cell is selected from the group consisting of mammalian and insect cells.
21. (Currently Amended) The ~~genetically engineered~~ microorganism according to claim 20, wherein the microorganism is *E. coli*.

Claims 22-26 (cancelled).

27. (withdrawn) A method for killing bacteria comprising contacting said bacteria with antibodies to an immunogenic molecule comprising a modified pneumolysin comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity in the presence of complement.
28. (withdrawn) The method according to claim 27, wherein the immunogenic molecule is a polysaccharide-polypeptide conjugate wherein the polysaccharide is a bacterial capsular polysaccharide.
29. (withdrawn) A method for immunization of mammals comprising administering a vaccine comprising the modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies

which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity and a pharmaceutically acceptable carrier to said mammals.

30. (withdrawn) A method for obtaining modified pneumolysin polypeptides, wherein said modified pneumolysin polypeptides have reduced hemolytic activity and are suitable for eliciting an immunogenetic response which is cross-reactive with wild-type pneumolysin comprising the steps of:

(a) mutating a nucleic acid molecule encoding wild-type pneumolysin to produce mutated nucleic acid molecules encoding modified pneumolysin polypeptides, wherein the modified pneumolysin polypeptides comprise one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 and expressing the mutated nucleic acid molecules in host cells;

(b) assaying the modified polypeptide expressed by the host cells for hemolytic activity; and

(c) identifying the modified pneumolysin polypeptides having substantially similar molecular weight as native wild-type pneumolysin and which are refoldable.

31. (Previously Presented) The molecule of claim 16 or claim 17, wherein the molecule is incorporated into a vector selected from the group consisting of a plasmid, cosmid, bacteriophage and yeast artificial chromosome.
32. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid sequence~~ of modified pneumolysin polypeptide pNVJ1.
33. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid sequence~~ of modified pneumolysin polypeptide pNVJ20.
34. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid sequence~~ of modified pneumolysin polypeptide pNVJ22.
35. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid sequence~~ of modified pneumolysin polypeptide pNVJ45.
36. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid sequence~~ of modified pneumolysin polypeptide pNVJ56.
37. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid sequence~~ of modified pneumolysin polypeptide pNV103.

38. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid~~
~~sequence of~~ modified pneumolysin polypeptide pNV207.
39. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid~~
~~sequence of~~ modified pneumolysin polypeptide pNV111.
40. (Currently Amended) A recombinant nucleic acid molecule encoding the ~~nucleic acid~~
~~sequence of~~ modified pneumolysin polypeptide pNV211.